

Claims

1. A partially-loaded antibody, comprising:
at least one interchain disulfide bond, and
at least two cytotoxic or cytostatic drugs or labels, each drug or label
5 conjugated to an interchain thiol;
wherein the points of the conjugation of the drug or label are readily
assignable.
2. The partially-loaded antibody of claim 1, wherein the antibody at
least four cytotoxic or cytostatic drugs, each drug conjugated to an interchain thiol.
- 10 3. The partially-loaded antibody of claim 1, which has the
configuration of species 4A or 4B.
4. The partially-loaded antibody of claim 1, which has the
configuration of species 4C or 4D.
- 15 5. The partially-loaded antibody of claim 1, which has the
configuration of species 4E.
6. The partially-loaded antibody of claim 1, which has the
configuration of species 4F.
7. The partially-loaded antibody of claim 1, wherein the antibody is a
humanized or chimeric antibody.
- 20 8. The partially-loaded antibody of claim 1, wherein the cytotoxic or
cytostatic agent is MMAF, MMAE, or AFP.
9. A pharmaceutical composition comprising the antibody of claim 1
and a pharmaceutically acceptable carrier.
- 25 10. An antibody, comprising:
at least one point of conjugation for a cytotoxic or cytostatic agent, wherein
the point of conjugation for the cytotoxic or cytostatic agent on the antibody can be readily

assigned, and wherein less than all possible points are conjugation are available for conjugation to the cytotoxic or cytostatic agent.

11. The antibody of claim 10, wherein the points of conjugation are interchain thiols.

5 12. The antibody of claim 10, wherein the point of conjugation is at least one of 4A through 4F.

13. The antibody of claim 10, wherein the point of conjugation is selected from at least one of the group consisting of 4A through 4F.

10 14. A composition of modified antibodies having assignable conjugation points, comprising:
at least four species of modified antibody, each species (a) having at least one specified conjugation pair having two interchain thiols and (b) at least one interchain disulfide bond.

15. The composition of claim 14, comprising a species 4A or 4B.

15 16. The composition of claim 14, comprising a species 4C or 4D.

17. The composition of claim 14, comprising a species 4E.

18. The composition of claim 14, comprising a species 4F.

19. The composition of claim 14, wherein the specified conjugation pair is at a constant light-constant heavy interchain disulfide bond.

20 20. The composition of claim 14, wherein the specified conjugation pair is at a constant heavy-constant heavy interchain disulfide bond.

21. The composition of claim 14, wherein the specified conjugation pair is proximal to the N-terminal end of the hinge region.

25 22. The composition of claim 14, wherein the specified conjugation pair is proximal to the C-terminal end of the hinge region.

23. The composition of claim 14, wherein the specified conjugation pair is at the constant light-constant heavy interchain sulfide bond and at the hinge region located closer to the N-terminal end of the modified antibody.

24. The composition of claim 14, wherein the specified conjugation pair is at the constant light-constant heavy interchain disulfide bond and at the hinge region located closer to the C-terminal end of the modified antibody.

25. The composition of claim 14, comprising at least two specified conjugation pairs at the constant light-constant heavy interchain disulfide bonds.

26. The composition of claim 14, comprising at least two specified conjugation pairs at the hinge region interchain disulfide bonds.

27. The composition of claim 14, further comprising a pharmaceutically acceptable carrier.

28. A partially loaded antibody, comprising:
at least one antigen-binding domain;
at least two reactive group on the antibody, and
at least two drugs or labels, each drug or label conjugated to a reactive group to form a point of conjugation;
wherein the points of conjugation for the drug or label are readily assignable.

29. A partially loaded, modified protein having assignable conjugation points, comprising:

a binding region for interaction with a binding partner,
at least two points of conjugation, each point of conjugation covalently linked a drug or label;

wherein less that all possible points of conjugation having a similar accessibility or activability are linked to a drug or label.

30. The modified protein of claim 29, wherein the protein is an antibody.

31. The modified protein of claim 29, wherein the points of conjugation are amino groups, vicinal hydroxyl groups, hydroxyl groups, carboxyl groups, or thiol groups.

32. The method of claim 29, wherein the protein is a receptor, a
5 receptor ligand, a hormone or a cytokine.

33. The use of a partially-loaded antibody comprising at least one interchain disulfide bond, and at least two cytotoxic or cytostatic drugs or labels, each drug or label conjugated to an interchain thiol; wherein the points of the conjugation of the drug or label are readily assignable, in the preparation of a medicament for the treatment
10 of cancer, immune disease, autoimmune disease or infectious disease.

34. A method of reducing and conjugating a drug to an antibody resulting in selectivity in the placement of the drug, comprising:
fully reducing the antibody with a reducing agent;
treating the fully reduced antibody with limiting amounts of a reoxidizing
15 agent to reform at least one interchain disulfide bond of the antibody, such that at least two interchain thiols remain; and
conjugating the drug to the interchain thiols.

35. The method of claim 34, wherein the reoxidizing agent is 5,5'-dithio-bis-2-nitrobenzoic acid, 4,4'-dithiodipyridine, 2,2'-dithiodipyridine, sodium
20 tetrathionate or iodosobenzoic acid.

36. The method of claim 34, wherein the drug is a cytotoxic or cytostatic agent or an immunosuppressive agent.

37. The method of claim 34, wherein the cytotoxic or cytostatic agent is a minor groove binder, AEB, or AEVB.

38. The method of claim 34, wherein the drug is MMAF, MMAE, or
25 AFP.

39. The method of claim 34, wherein the reducing agent is DTT or TCEP.

40. A method of reducing antibody interchain disulfide bonds and conjugating a drug to the resulting interchain thiols resulting in selectivity in the placement of the drugs on the antibody, comprising:

5 fully reducing the antibody with a reducing agent to form interchain thiols;
partially reoxidizing the antibody with a reoxidizing agent to reform at least one interchain disulfide bond; and
conjugating the drug to the interchain thiols.

41. The method of claim 40, wherein the reoxidizing agent is 5,5'-dithio-bis-2-nitrobenzoic acid, 4,4'-dithiodipyridine, 2,2'-dithiodipyridine, sodium
10 tetrathionate, or iodosobenzoic acid.

42. The method of claim 40, wherein the reducing agent is DTT or TCEP.

43. The method of claims 40, further comprising purifying the partially reoxidized antibody.

15 44. The method of claim 40, wherein the drug is MMAF, MMAE, or AFP.

45. A method of reducing antibody interchain disulfide bonds and conjugating a drug to the resulting interchain thiols to selectively locate drugs on the antibody, comprising:

20 partially reducing the antibody with a reducing agent to form at least two interchain thiols; and
conjugating the drug to the interchain thiols of the partially reduced antibody.

46. The method of claim 45,
25 wherein the antibody is partially reduced with a limiting concentration of a reducing agent in a buffer with a chelating agent; and
wherein the drug is conjugated by cooling the antibody solution and dissolving the drug in a cold solvent and mixing with the antibody solution;

allowing the antibody and drug solution to incubate for a period of time sufficient to form an antibody-drug conjugate;

quenching the excess drug with a thiol-containing reagent; and

purifying the resulting conjugate.

5 47. The method of claim 46, wherein the antibody is partially reduced for about 1 hour at about 37 °C.

 48. The method of claim 46, wherein the reduced antibody is cooled to about 0 °C.

10 49. The method of claim 46, wherein the antibody and drug solution are incubated for about 30 minutes at about 0 °C.

 50. The method of claim 45, wherein the thiol-containing reagent is cysteine or N-acetyl cysteine.

 51. The method of claim 45, wherein the reducing agent is DTT or TCEP.

15 52. The method of claim 46, wherein the buffer is a sodium borate solution and the chelating agent is diethylenetriaminepentaacetic acid.

 53. The method of claim 46, wherein the chelating agent is diethylenetriaminepentaacetic acid or EDTA.

20 54. The method of claim 45, further comprising purifying the reduced antibody.

 55. The method of claim 54, wherein the reduced antibody is purified using column chromatography, dialysis, or diafiltration.

 56. The method of claim 55, wherein the column is a desalting column.

25 57. The method of claim 57, wherein the desalting column is a PD-10 column.

58. The method of claim 45, wherein the reduced antibody is not purified after partial reduction and prior to conjugation.

59. The method of claim 45, wherein the conjugate is purified using column chromatography, dialysis, or diafiltration.

5 60. The method of claim 59, wherein the column is a desalting column.

61. The method if claim 61, wherein the desalting column is a PD-10 column.

62. The method of claim 45, wherein the solvent is acetonitrile, alcohol or DMSO.

10 63. The method of claim 45, wherein the drug is a cytotoxic or a cytostatic agent.

64. A method of producing an antibody with selective conjugation of drug comprising:

15 fully reducing the antibody for a period of time sufficient to produce interchain thiols, as determined by DTNB titration, by adding a large excess of a reducing agent and incubating the solution at about 37 °C for about 30 minutes;

purifying the antibody;

partially reoxidizing the antibody using an oxidizing agent to form at least one interchain disulfide bond by

20 cooling the reduced antibody to 0 °C;

treating the reduced and cooled antibody with 1.5 to 2.5 molar equivalents of the oxidizing agent;

mixing the solution by inversion;

allowing the solution to incubate at about 0 °C for about 10 minutes;

25 purifying the partially reoxidized antibody;

conjugating the drug to the interchain thiols of the partially reoxidized antibody to form a conjugated antibody; and

purifying the conjugated antibody.

65. The method of claim 64, wherein the reducing agent is DTT or TCEP.

66. The method of claim 64, wherein the antibody is purified using column chromatography, dialysis, or diafiltration.

5 67. The method of claim 66, wherein the column is a desalting column.

68. The method of claim 67, wherein the desalting column is a PD-10 column.

69. The method of claim 64, wherein the conjugated antibody is purified using column chromatography, dialysis, or diafiltration.

10 70. The method of claim 69, wherein the column is a desalting column.

71. The method if claim 70, wherein the desalting column is a PD-10 column.

15 72. The method of claim 64, wherein the reoxidizing agent is 5,5'-dithio-bis-2-nitrobenzoic acid, 4,4'-dithiodipyridine, 2,2'-dithiodipyridine, sodium tetrathionate, or iodosobenzoic acid.

20 73. A method of preparing a conjugate of a protein having one or more disulfide bonds, and a drug reactive with free thiols, which comprises:
partially reducing the protein with a reducing agent; and
conjugating the drug reactive with free thiols to the partially reduced protein.

74 A method of preparing a conjugate of a protein having one or more disulfide bonds, and a drug reactive with free thiols, which comprises:

fully reducing the protein with a reducing agent;

5 partially reoxidizing the protein with a reoxidizing agent; and

conjugating the drug reactive with free thiols to the antibody.

75. A method of forming a partially loaded antibody, comprising:

providing a solution containing an antibody,

10 adjusting the pH of the antibody solution to about pH 7.5 and adding a chelating agent;

heating the antibody solution to about 37 °C;

adding a molar excess of TCEP to the antibody solution and reacting for a sufficient period of time at about 37 °C to partially reduce the interchain disulfide groups of the antibody to form interchain thiols;

15 cooling the antibody solution to between about 2-8 °C;

conjugating the drug to the interchain thiols of the partially reduced antibody by adding a slight molar excess of the drug to the antibody solution and reacting for a sufficient period of time to form the partially loaded antibody; and

purifying the partially loaded antibody.

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